

Interactive comment on “Seasonal occurrence of anoxygenic photosynthesis in Tillari and Selaulim reservoirs, Western India” by S. Kurian et al.

By Dr. Rutger De Wit

Referee’s comment:

The current paper seems technically sound, except perhaps the apparent lack of LCMS/MS, see below. But, it lacks sufficient depth in presentation of data and interpretation and calls for extensive revision.

Reply:

We thank Dr. De Wit for his critical review which has helped in improving the manuscript to a considerable extent. We have tried our best to address the issues raised by him. However, since we are not left with samples any more, additional analyses could not be performed, and the identification of bacteriochlorophylls is restricted based on the available data set (HPLC and LC/MS) and published literature. The detailed response to referee’s comments is as follows.

Referee’s comment:

The reservoirs described in this paper are clearly “monomictic”, i.e. one mixing period and one stratification period per year. They should use this common limnological terminology in addition to the term “meromictic” (permanently stratified) used for other systems.

Reply: Based on our monthly observations in the Tillari Reservoir (unpublished data), the water column gets vertically mixed twice in a year. Hence it is described as ‘dimictic’ in the revised manuscript.

Referee’s comment:

Lines 9-10 page 12155: Note that green sulfur bacteria and filamentous green non-sulfur bacteria (Chloroflexaceae) always contain minor amounts of BChl a (compared to BChl c,d, e). The BChl a has a fundamental role in the photosynthetic reaction (RC) and in green sulfurs also occurs in the base plate of the chlorosome.

Reply: Mentioned in the introduction of the revised manuscript. But we could not detect BChl a in our samples and the major peaks were identified as BChl e isomers.

Referee’s comment:

Line 15 page 12155: The authors of this paper do not acknowledge the important contribution of Airs et al. (2001a,b, 2002) in the laboratory of Brendan Keely (York University), who were first to develop and apply LC-MS techniques for Bacteriochlorophyll analyses in cultures and lake water samples.

Reply: We regret the omission and thank the referee for pointing it out. These references are cited in the revised manuscript.

Referee’s comment:

Detail on Bacteriochlorophylls is absolutely insufficient. Rather than designating simply BChl-e1, BChl-e2 and BChl-e3 as 15 years ago, the authors should carefully interpret their LC-MS spectra and probably apply Tandem mass spectrometry (LC-MS/MS) to assign bacteriochlorophyll structures from their fragmentation during MS/MS analysis (Airs et al., 2001b, Airs & Keely, 2002). Nowadays, it is now possible to describe with great detail the substituents at position C8 and C12 (C2, C3, C4 units) and the chemical nature of the alcohol esterified at position C17. It is particularly important to report whether this alcohol is an isoprenoid (often farnesol) or straight chain, in all cases indicate length etc.

Reply: The analysis was carried out on HPLC and LC-MS (single quadrupole) using an APCI source operated in the positive mode. The samples were run in full scan and also selective ion monitoring (SIM) mode. Based on the available spectra and published literature (Airs et al., 2001 and Glaeser et al., 2002, Massè et al, 2004 etc.) the bacteriochlorophyll e isomers are identified as farnesyl esterified BChl e2 (Et, Et) m/z=821.5; e3 (n-Pr, Et) m/z=835.5 and e4 (i-Bu, Et) m/z=849.5. Hence identification of alkyl substituents and nature of alcohol esterified on the tetrapyrrole ring are based on LC/MS spectra and results of the previous studies, as Tandem Mass Spectrometry was not available for present study. However, our major objective is to report the occurrence of anoxygenic photosynthesis in Indian reservoirs, which was not reported earlier.

Referee's comment:

A bit vaguely, the authors suggest that different highly alkylated allomers are long wavelength absorbing. Perhaps the authors refer the following suggestion that the different C-8 and C-12 side chains on the bacteriochlorophylls modify the aggregation and therefore the packing of structures in the chlorosomes, with an impact on the in vivo absorption spectrum (Airs et al., 2001b), which might provide a basis for a cellular response to light limitation (Borrego et al., 1998). However, to my point of view this remains still enigmatic, because it would require matching the BChl composition of the extracted cells with their in vivo spectrum in the living cells.

Reply: The respective section has been modified to address the referee's concern.

Referee's comment:

The authors also missed our publication (Massé et al., 2004) where we discussed the drivers that could determine whether esterification of isoprenoid or straight-chain alcohols is favored at position C17. Hence we (Massé et al., 2004) reported that in cultured *Chlorobium phaeobacteroides*, the proportion of straight alkyl chains over isoprenoid esterified side chains shifted markedly with increasing light intensity: the isoprenoid side chains dominated at low light intensities, while the straight-chain alkyl substituents dominated at higher light intensities. We proposed that this phenomenon may be explained as a result of changing availability of reducing power, i.e. the highly reduced straight-chain alcohols have a higher biosynthetic demand for NADPH₂ than the polyunsaturated isoprenoid with the same number of carbon atoms. Please carefully consider all these points for your data interpretation and discussion.

Reply: Accepted. This work is now cited in the revised manuscript (Section 4.2).

Referee's comment:

Finally, the authors engage a comparison of standing stocks (which is the right term indeed) of green sulfur bacteria compared to oxygenic phototrophs. However, this does not allow to make conclusions about the productivity of both groups, because no C-fixation rates have been measured. To me it appears very likely that the low oxygenic phototrophic biomass is the result of growth and grazing, while grazing on the green sulfur bacteria in the anoxic part is less likely or at least less intense. Hence, the high standing stock of green sulfur bacteria may be the result of slow accumulation of biomass not requiring high productivity rates.

Reply: We agree with the referee. The oxygenic photosynthesis has to be more important than anoxygenic photosynthesis because the development of anoxic conditions is the consequence of the former. We have no data either on grazing rates or production rates, but a comparison of standing stocks is still interesting. However, our zooplankton analysis during June, 2010 showed Daphnia as the dominant grazers. Section 4.3 has been modified to some extent in order to accommodate the referee's view.